PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
2026-4236PC		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 97/ 14306	14/08/1997	14/08/1996
Applicant		
NATIONAL INSTITUTES OF HEALTH et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 5 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [X] **Certain claims were found unsearchable** (see Box I).

2. [ ] **Unity of invention is lacking** (see Box II).

3. [X] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
   - [X] filed with the international application.
   - [ ] furnished by the applicant separately from the international application,
     - [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
   - [ ] Transcribed by this Authority

4. With regard to the **title,**
   - [ ] the text is approved as submitted by the applicant.
   - [X] the text has been established by this Authority to read as follows:

   A VECTOR FOR POLYNUCLEOTIDE VACCINES

5. With regard to the **abstract,**
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this International Search Report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:

   Figure No. 1
   - [X] as suggested by the applicant.
   - [ ] because the applicant failed to suggest a figure.
   - [ ] because this figure better characterizes the invention.
   - [ ] None of the figures.

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

United States Patent and Trademark Office
(Box PCT)
Crystal Plaza 2
Washington, DC 20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 09 March 1998 (09.03.98)	
International application No. PCT/US97/14306	**Applicant's or agent's file reference** 2026-4236PC
International filing date (day/month/year) 14 August 1997 (14.08.97)	**Priority date** (day/month/year) 14 August 1996 (14.08.96)
Applicant NELSON, Edward, L. et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

09 February 1998 (09.02.98)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Ting Zhao

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
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EE	Estonia	**LR**	Liberia	**SG**	Singapore		

INTERNATIONAL SEARCH REPORT

Interr ıal Application No
PCT/US 97/14306

A. CLASSIFICATION F SUBJECT MATTER
IPC 6 C12N15/85 A61K48/00 C12N5/10 C07K16/32 C07K16/30
C12Q1/68 C12N15/11

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N A61K C07K C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 07347 A (BIO RAD LABORATORIES) 16 March 1995	49
Y	see page 2, line 36 - page 4, line 37 see page 7, line 8 - line 32 see page 8, line 31 - page 9, line 14 see page 10, line 9 - line 35 see page 12, line 12 - page 14, line 2; example 2 --- -/--	1-5,17, 24,25, 36,37

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

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Date of the actual completion of the international search
17 November 1997

Date of mailing of the international search report
27. 11. 97

Name and mailing address of the ISA
European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer
Montero Lopez, B

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter: nal Application No
PCT/US 97/14306

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	COLOMA, M. JOSEFINA ET AL: "Novel vectors for the expression of antibody molecules using variable regions generated by polymerase chain reaction" J. IMMUNOL. METHODS (1992), 152(1), 89-104 CODEN: JIMMBG;ISSN: 0022-1759, 1992, XP000289684	49
Y	see abstract see page 90, right-hand column, paragraph 2 see page 92, right-hand column, paragraph 3 - right-hand column, paragraph 3 ---	1-5
X	WO 92 01055 A (BOEHRINGER INGELHEIM INT) 23 January 1992 see page 4, paragraph 2 - page 5, paragraph 1; examples 1-5 ---	49
Y	PETER J. NELSON ET AL.: "Genomic organization and transcriptional regulation of the RANTES chemokine gene" JOURNAL OF IMMUNOLOGY, vol. 151, no. 5, 1 September 1993, BALTIMORE US, pages 2601-2612, XP002047102 cited in the application see abstract see page 2601, right-hand column, paragraph 2 - page 2602, left-hand column, paragraph 1 see page 2603, right-hand column, paragraph 2 - page 2604, left-hand column, paragraph 1 see page 2608, left-hand column, paragraph 2 - page 2610, left-hand column, paragraph 1 -----	1-5,17, 24,25, 36,37

2

INTERNATIONAL SEARCH REPORT

ınational application No.
PCT/US 97/14306

Box I Obs rvations wher certain claims were found unsearchable (Continuation f it m 1 f first sh et)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Remark : Although claims 38-45 are directed to a method of treatment of the human/animal body , the search has been carried out and based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

...formation on patent family members

Interr ...al Application No
PCT/US 97/14306

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9507347 A	16-03-95	US 5426039 A	20-06-95
		CA 2171096 A	16-03-95
		EP 0722487 A	24-07-96
		JP 9502350 T	11-03-97
WO 9201055 A	23-01-92	DE 4021917 A	16-01-92
		DE 4035877 A	14-05-92
		AU 650893 B	07-07-94
		AU 8208291 A	04-02-92
		DE 59101397 D	19-05-94
		EP 0538300 A	28-04-93
		ES 2063515 T	01-01-95
		HU 65846 A	28-07-94
		JP 6502987 T	07-04-94
		SK 386392 A	10-08-94

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PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (PCT/IPEA/416)
2026-4236PC		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US97/14306	14/08/1997	14/08/1996
International Patent Classification (IPC) or national classification and IPC		
C12N15/85		
Applicant		
NATIONAL INSTITUTES OF HEALTH et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 10 sheets.

3. This report contains indications relating to the following items:

I	☒	Basis of the report
II	☒	Priority
III	☒	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain documents cited
VII	☐	Certain defects in the international application
VIII	☒	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
09/02/1998	17. 11. 98
Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	Julia, P Telephone No. (+49-89) 2399-8410

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US97/14306

I. Basis of th r port

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-95 as originally filed

Claims, No.:

1-6,8-30,32-48,50-62, 64-69 with telefax of 09/10/1998

Drawings, sheets:

1/8-8/8 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☒ the claims, Nos.: 7, 31, 49, 63
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

II. Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

   ☐ copy of the earlier application whose priority has been claimed.

   ☐ translation of the earlier application whose priority has been claimed.

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has

been found invalid.

Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

see separate sheet

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 24-27, 38-45.

because:

☒ the said international application, or the said claims Nos. 24-27, 38-45 relate to the following subject matter which does not require an international preliminary examination (*specify*):

see separate sheet

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-6, 8-23, 28-30, 32-37, 46-47, 52-62, 64-69
	No:	Claims	48, 50-51
Inventive step (IS)	Yes:	Claims	1-6, 8-23, 28-30, 32-37, 46-47, 55-62, 64-69
	No:	Claims	48, 50-54
Industrial applicability (IA)	Yes:	Claims	1-6, 8-23, 28-30, 32-37, 46-48, 50-62, 64-69
	No:	Claims	

2. Citations and explanations

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

1. Additional remarks to item I :

The application refers to the presence of "minimal exogenous non-human components" and that ".. the vector does not contain sequences which may increase inactivation by methylation or changes in tertiary structure .." (page 12, lines 13-20 and page 25, lines 22-24) as well as that the ".. vector does not contain foreign antibiotic resistance genes .." (page 14, lines 38-39). The Applicant, however, has failed to provide any basis for the specific wording ".. lacking nucleic acid sequences encoding vector-derived polypeptides ..". Page 20, lines 10-15 of the application refers to general chemokines and cytokines but there is no mention of the specific chemokine "IP-10". These amendments are considered to go beyond the disclosure as originally filed (Rule 70.2 (c) PCT).

2. Additional remarks to item II :

This international preliminary examination report (IPER) has been done considering the priority date 14.08.96 as a valid date.

3. Additional remarks to item III :

The subject matter of **claims 24-27** is directed to a method for expressing at least one target antigen or antigenic epitope thereof **in cells** comprising the introduction of the disclosed "humanized polynucleotide vector" in said cells. Said method includes the **in vivo** introduction of said vector into said cells (page 8 lines 4-7, page 22 line 32 - page 24 line 19, etc..). Thus, as far as the claimed subject matter is not clearly limited to an "ex vivo" method, the IPEA considers that the claimed method embraces a method of treatment of the human or animal body and thus, it is excluded from examination by Article 34(4)(a)(i) PCT in combination with Rule 67(iv) PCT. The same objection applies for the subject matter of **claims 38-45**. The attention of the Applicant is also drawn to the fact that for such a subject matter no unified criteria exist in PCT for the assessment whether it is industrially applicable or not. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject matter of claims to the use of a compound in medical treatment, but will allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

4. Additional remarks to item V :

The present application discloses a humanized polynucleotide vector comprising a human derived promoter or mammalian homolog thereof (440 base pairs of the human promoter

RANTES) which is functional in target tissue or target cells (myocytes and professional antigen presenting cells), said promoter being operably linked to a "sequence acceptance site" (Figure 2) which directionally accepts cDNA target products from reverse-transcriptase PCR (rtPCR) cloning via unique sites within an interrupted palindrome recognition sequence for a restriction endonuclease (Bgl I), said vector having minimal exogenous non-human DNA components and in particular without any foreign antibiotic resistance gene (i.e. lacking nucleic acid sequences encoding vector-derived polypeptides) and wherein said cDNA target products are defined as target antigens (products of a tumour associated genetic derangement, tumour antigen such as p53, RB, ras, etc.. bacterial, viral or parasitic antigens, etc..) or antigenic epitopes thereof alone or in combination with a cytokine (IL2, IL3, GM-CSF, etc...) or chemokine (RANTES, MCP, defensins, etc...). The application further discloses a related humanized polynucleotide vector vaccine, pharmaceutical compositions, kits, antibodies, methods for expressing at least one target antigen, uses of said vectors, etc...

The following documents have been cited in the International Search Report as being relevant for assessing the novelty and inventiveness of the claimed subject matter :

i) M.J. Coloma et al., J. Immunol. Methods 1992, Vol. 152, pages 89-104 (**D1**) discloses different vectors for expressing immunoglobulin variable regions cloned by PCR. These (polynucleotide) vectors comprise a mammalian (murine) homolog of a human derived promoter (Vh promoter) which is functional in a target cell (myeloma cells) and it is operably linked to a "sequence acceptance site" which directionally accepts cDNA target products from rtPCR cloning (Ig variable regions) via unique sites within an (uninterrupted) palindrome recognition sequence for a restriction endonuclease (NheI, BglII and EcoRV sites). These vectors further comprise a nucleic acid sequence encoding an antibiotic resistance (and eukaryotic selection markers). There are at least two technical differences between the disclosure of **D1** and the claimed "humanized polynucleotide vectors", namely (**a**) the presence of vector-derived polypeptides (antibiotic resistance and eukaryotic selection markers) in **D1** and (**b**) the fact that the palindrome recognition sequence is uninterrupted in **D1**. Thus, the claimed "humanized polynucleotide vectors" are considered to be novel under Article 33 (2) PCT (see however paragraph (3) under "Additional remarks to item VIII" for clarity considerations in respect of the selection markers).

The application further demonstrates the lower toxicity of the claimed "humanized

polynucleotide vectors" due to the presence of minimal exogenous DNA and of being maximally "humanized" (i.e. absence of any foreign open reading frame (ORF) or lacking any nucleic acid sequence encoding vector-derived polypeptides and in particular any antibiotic resistance encoding nucleic acid sequence). Furthermore, the presence of an interrupted palindrome recognition sequence offers several advantages for cloning rtPCR products over the vectors disclosed in **D1** (lower likelihood of cloning an incomplete rtPCR fragment). Thus, the IPEA considers that the "humanized polynucleotide vectors" fulfil the requirements of Article 33 (3) PCT.

ii) the document P.J. Nelson et al., J. Immunol. 1993, Vol.151, pages 2601-2612 (**D2**) has been cited in the ISR for RANTES promoters and functional portions thereof. However, there is no reference to any "humanized polynucleotide vector".

iii) WO-A-95/07347 (**D3**) emphasizes the importance and difficulties encountered for cloning PCR products including the incorporation of restriction sites into the PCR primers (page 2). **D3** discloses a method for cloning cDNA target products from rtPCR into a polynucleotide vector using any restriction endonuclease which generates 5' overhangs at the site of cleavage and wherein said vector, containing one or more restriction endonuclease cleavage sites, is capable of replication in a bacterial cell and optionally in an eukaryotic cell (shuttle vector). **D3** refers to general vectors (page 10) and it is exemplified by using the well-known and commercially available pTZ18(U). The target cDNA products are further defined as being proteins which serve as diagnostic markers for genetic mutations (page 5 line 37-page 6 line 3). There is however no explicit mention of the features characterizing the claimed "humanized polynucleotide vector" (presence of minimal exogenous DNA, human derived promoter, sequence acceptance site with unique sites within an interrupted palindrome recognition sequence for a restriction endonuclease, etc...).

iv) WO-A-92/01055 (**D4**) discloses different vectors for expressing and producing O-glycosylated interferon-alpha, wherein parts of said vectors have been produced by PCR amplification. None of the vectors disclosed comprise the elements required in **claim 1**, namely a human derived promoter, sequence acceptance site with unique sites within an interrupted palindrome recognition sequence for a restriction endonuclease, etc.. Thus, its content is not considered to be relevant for the claimed subject matter.

Thus, the subject matter of **claims 1-6, 8-23, 28-30, 32-37, 46-47, 55-62 and 64-69** fulfil the requirements of Articles 33 (2) and (3) PCT.

v) The subject matter of **claim 48** is directed to an antibody specific for a target antigen present in the claimed humanized polynucleotide vector vaccine (and expressed by the mammalian target tissue or cell). The antibody is, however, defined against any target antigen (and not against the humanized polynucleotide vector) and, as far as said antigen is not clearly defined, said wording includes known antigens and thus, it comprises known antibodies raised against said known target antigens using other vectors (if at all !!) and/or other immunization systems or methods. This subject matter is not considered to fulfil the requirements of Articles 33 (2), (3) PCT.

vi) **claim 50** is a "product-claim" directed to a nucleotide sequence "per se" and that, apart from its intended use (namely directionally accepting cDNA target products from rtPCR cloning), the actual technical features defining said nucleotide sequence are the presence of a restriction site within an interrupted palindrome recognition sequence. However, nucleotide sequences comprising such technical features are well known in the prior art (see HindIII fragment in **D1**) and said sequences are certainly suitable for being used as "sequence acceptance sites" in the sense of the present application. Thus, the IPEA considers that the subject matter of **claim 50-51** does not fulfil the requirements of Article 33 (2) and (3) PCT. Furthermore, as far as no specific advantage has been demonstrated for the specific "sequence acceptance site" of the application over other alternative and well known nucleotide sequences of the prior art, the IPEA considers that the claimed sequences only represent an "arbitrary selection" among all other sequences available and thus, the subject matter of **claims 52-54** does not fulfil the criteria of Article 33 (3) PCT.

3. Additional remarks to item VIII :

The following objections are also raised under Article 6 PCT concerning the clarity of the claims :

1) references to specific nucleotide sequences (RANTES, colE1, etc...), length of said sequences and portions thereof (440 base pairs, 635 base pairs, etc...) as well as general features of said sequences (NCO site, etc..) but without further indicating the corresponding SEQ ID No. are ambiguous and can not be seen as a complete and clear

disclosure. In this respect, the attention of the Applicant is also drawn to the fact that all abbreviations used (Tre17, NF1, FAP, ROS, FIS, etc..) must be well known in the prior art, without implying any possible ambiguity (i.e. unique abbreviations) and easily available to the skilled person (bibliographic references), otherwise the use of such abbreviations does not fulfil the requirements of Article 6 PCT and reference must be made to a specific SEQ ID no.

2) the subject matter of **claim 14** is directed to (**1**) SEQ ID No.: 16, which according to pages 29-30 corresponds to the base vector pITL (Figures 1 and 5), (**2**) SEQ ID No.: 27, which according to pages 56-57 corresponds to the polynucleotide vector pITL-A (ATCC designation 98401) and (**3**) SEQ ID No.: 28, which according to page 55 corresponds to the polynucleotide vector pITL-1 (ATCC designation 98400). The same subject matter is also claimed in **claim 15** (polynucleotide vectors contained in ATCC 98400 and 98401), which thus is seen as redundant.

3) the meaning of the wording ".. lacking nucleic acid sequences encoding vector-derived polypeptides .." is considered to be ambiguous as far as said polypeptides are not clearly defined. The description refers to a "minimal exogenous non-human components" but contemplates the presence of certain non-human derived genes too, such as the nucleic acid sequences coding for antibiotic resistance (explicitly disclaimed in the claims) and/or sequences providing mechanisms for selection and growth of the recombinant plasmids in bacteria or yeast (page 14, lines 36-39).

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 15/85, A61K 48/00, C12N 5/10, C07K 16/32, 16/30, C12Q 1/68, C12N 15/11

A1

(11) International Publication Number: **WO 98/06863**

(43) International Publication Date: 19 February 1998 (19.02.98)

(21) International Application Number: PCT/US97/14306

(22) International Filing Date: 14 August 1997 (14.08.97)

(30) Priority Data:
60/023,931 14 August 1996 (14.08.96) US

(60) Parent Application or Grant
(63) Related by Continuation
US 60/023,931 (CIP)
Filed on 14 August 1996 (14.08.96)

(71) Applicant *(for all designated States except US)*: THE GOVERNMENT OF THE UNITED STATES OF AMERICA, represented by THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES [US/US]; Office of Technology Transfer, National Institutes of Health, Suite 325, 6011 Executive Boulevard, Rockville, MD 20852 (US).

(72) Inventors; and
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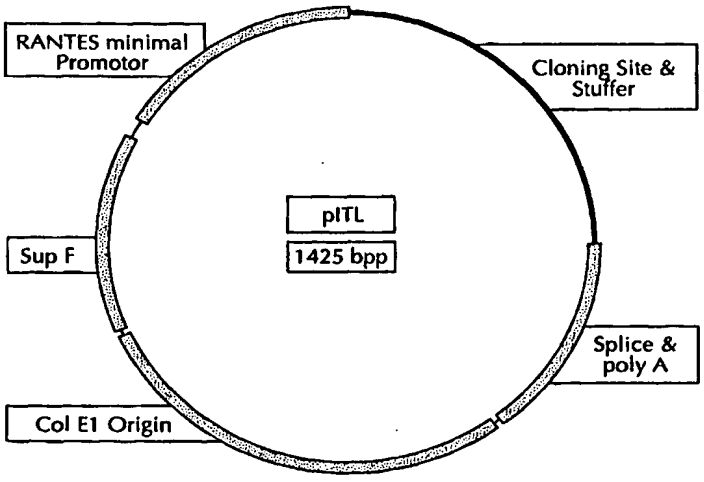

(57) Abstract

The invention is a "humanized" polynucleotide vector vaccine which uses covalent closed circular plasmid DNA, "naked DNA", to express target antigens. The vector is non-replicating in mammalian cells but is capable of extended stable expression of the target sequences in skeletal muscle and professional antigen presenting cells generating an immune response to the target antigen in immunized individuals. The polynucleotide vector is particularly useful in accommodating monomorphic and polymorphic nucleic acid sequences encoding tumor antigens via PCR technology.

1. A humanized polynucleotide vector comprising:

a human derived promoter or mammalian homolog thereof which is functional in a target tissue or target cells, said promoter operably linked to a sequence acceptance site which directionally accepts cDNA target products from rtPCR cloning via unique sites within an interrupted palindrome recognition sequence for a restriction endonuclease, said vector lacking nucleic acid sequences encoding vector-derived polypeptides wherein, said vector lacks an antibiotic resistance encoding nucleic acid sequence.

2. The humanized polynucleotide vector according to claim 1 wherein the target cells are selected from the group consisting of myocytes and professional antigen presenting cells.

3. The humanized polynucleotide vector according to claim 1 or 2 wherein the target cells or target tissue are human.

4. The humanized polynucleotide vector according to claims 1-3 wherein the human derived promoter is a RANTES promoter or portion thereof.

5. The humanized polynucleotide vector according to claim 4 wherein the promoter has approximately 440 base pairs.

6. The humanized polynucleotide vector according to claims 4 or 5 wherein the portion corresponds to a region spanning the NCO site through the KpnI site of the genomic RANTES promoter

8. The humanized polynucleotide vector according to claims 1-5 or 6 further comprising an origin for replication and growth and a nucleic acid sequence which allows for selection of recombinant plasmids.

9. The humanized polynucleotide vector according to claim 8 wherein the origin for replication is colE1 or functional portion thereof.

10. The humanized polynucleotide vector according to claim 8 wherein the origin for replication comprises a 635 base pair region of the colE1 origin of replication.

11. The humanized polynucleotide vector according to claim 1 to 6 or 8-10 further comprising a human-derived 3' splice sequence and a human-derived poly A sequence, both sequences located downstream of the sequence acceptance site.

12. The humanized polynucleotide vector according to claim 11 wherein the human derived 3' splice and poly A sequence are derived from human growth hormone.

13. A polynucleotide vector according to claims 1-6 or 8-12 wherein a 5' sequence acceptance site reads on the positive strand as GCCACCATGGCC.

14. A polynucleotide vector comprising SEQ ID No 16, SEQ ID No 27 or SEQ ID No 28.

15. A polynucleotide vector contained within a host cell deposited with the ATCC under the ATCC designation 98400 or ATCC designation 98401.

16. A polynucleotide vector according to claims 1-6 or 8-15 further comprising cDNA target products, and an optional internal ribosomal entry site, said cDNA target products integrated into said sequence acceptance site, said cDNA target products comprising a nucleotide sequence encoding at least one target antigen or antigenic epitope thereof alone or in combination with a nucleotide sequence encoding a cytokine or chemokine.

17. A polynucleotide vector vaccine comprising a human derived promoter or mammalian homolog thereof which is functional in a mammalian target tissue or mammalian target cell, said promoter operably linked to a sequence

acceptance site which directionally accepts cDNA target products from rtPCR cloning via unique sites within an interrupted palindrome recognition sequence for a restriction endonuclease, an optional internal ribosomal entry site, and cDNA target products, said cDNA target products integrated into said sequence acceptance site, said cDNA target products comprising a nucleotide sequence encoding at least one target antigen or antigenic epitope thereof, and said vector lacking nucleic acid sequences encoding vector-derived polypeptides wherein, said vector lacks an antibiotic resistance encoding nucleic acid sequence.

18. A polynucleotide vector vaccine according to claim 17 wherein the target antigen is a product of a tumor associated genetic derangement.

19. A polynucleotide vector vaccine according to claim 17 wherein the target antigen is a tumor antigen, bacterial antigen, viral antigen, or parasitic antigen.

20. The polynucleotide vector vaccine according to claims 17 or 18, wherein the tumor antigen is p53, RB, ras, int-2, Hst, Tre17, BRCA-1, BRCA-2, MUC-1, HER-2/neu, truncated EGFRvIII, CEA, MyC, Myb, OB-1, OB-2, BCR/ABL, GIP, GSP, RET, ROS, FIS, SRC, TRC, WT1, DCC, NF1, FAP, MEN-1, ERB-B1 or combinations thereof.

21. A polynucleotide vector vaccine according to claim 17, 18, 19 or 20 further comprising an additional cDNA target product comprising a nucleic acid sequence encoding a cytokine or chemokine.

22. A polynucleotide vector vaccine according to claim 21 wherein the cytokine is selected from the group consisting of interleukin 2, interleukin 3, interleukin 4, interleukin 7, interleukin 8, interleukin 12, interleukin 15, GM-CSF, tumor necrosis factor, and interferon.

23. A polynucleotide vector vaccine according to claim 21 wherein the chemokine is selected from the group consisting of RANTES, MCP, MIP-1α, MIP-1β, defensins, IP-10 and combinations thereof.

24. A method for expressing at least one target antigen or antigenic epitope thereof in cells comprising:

introducing a humanized polynucleotide vector into said cells, under conditions for expression of the target antigen or antigenic epitope thereof, said vector comprising:

a human derived promoter or mammalian homolog thereof, which is functional in said cells, said promoter operably linked to a sequence acceptance site which directionally accepts cDNA target products from rtPCR cloning via unique sites within an interrupted palindrome recognition sequence for a restriction endonuclease and,

cDNA target products, and an optional internal ribosomal entry site, said cDNA target products integrated into said sequence acceptance site, said cDNA target products comprising a nucleic acid sequence encoding at least one target antigen or antigenic epitope thereof, and said vector lacking nucleic acid sequences encoding vector-derived polypeptides , wherein said vector lacks an antibiotic resistance encoding nucleic acid sequence.

25. The method of claim 24 wherein the cells are selected from the group consisting of myocytes and professional antigen presenting cells.

26. The method of claim 24 wherein the target antigen is a tumor antigen bacterial antigen, viral antigen, or parasitic antigen.

27. The method of claim 26 wherein the tumor antigen is p53, RB, ras, int-2, Hst, Tre 17, BRCA-1, BRCA-2, MUC-1, HER-2/neu, truncated EGFRvIII, CEA, MyC, Myb, OB-1, OB-2, BCR/ABL, GIP, GSP, RET, ROS, FIS, SRC, TRC, WT1, DCC, NF1, FAB, MEN-1, ERB-B1 or combinations thereof.

28. A pharmaceutical composition comprising at least one polynucleotide vector according to claims 1-6 or 8-16 and a pharmaceutically acceptable carrier.

29. A pharmaceutical composition comprising the polynucleotide vector vaccine according to claims 17-22 or 23 and a pharmaceutically acceptable carrier.

30. A kit comprising the polynucleotide vector according to claims 1-6 or 8-16.

32. A kit comprising the polynucleotide vector vaccine according to claims 17-22 or 23.

33. A kit according to claim 32, further comprising an expression enhancing agent.

34. The kit according to claim 33 wherein the expression enhancing agent

is a mycotoxic agent.

35. The kit according to claim 34 wherein the mycotoxic agent is bupivacaine-HCl and dextrose.

36. A host cell comprising:

the polynucleotide vector of claim 17-22 or 23, wherein the host cell is capable of expressing the target antigen or antigenic epitope.

37. The host cell according to claim 36 wherein the host cell is a myocyte or professional antigen presenting cell.

38. A method of stimulating a specific immune response to at least one target antigen or antigenic epitope thereof in a mammal comprising: administration of an effective amount of a polynucleotide vector vaccine according to claim 17-22 or 23 into the mammal, said amount elicits the specific immune response to the target antigen or epitope thereof.

39. The method according to claim 38, wherein a site of administration is muscle or skin.

40. The method according to claim 38 further comprising administration of effective amount of an expression enhancing agent prior to administration of the polynucleotide vector vaccine.

41. The method according to claim 40 wherein the expression enhancing agent is a mycotoxic agent.

42. The method according to claim 41 wherein the mycotoxic agent is bupivacaine-HCl or dextrose.

43. The method according to claim 38-41 or 42 wherein the target antigen is a tumor antigen, bacterial antigen, viral antigen or parasitic antigen.

44. The method according to claim 43 wherein the tumor antigen is selected from the group consisting of P53, RB, ras, int-2, Hst, Tre 17, BRCA-1, BRCA-2, MUC-1, HER-2/neu, truncated EGFRvIII, CEA, MyC, Myb, OB-1, OB-2, BCR/ABL, GIP, GSP, RET, ROS, FIS, SRC, TRC, WT1, DCC, NF1, FAB, MEN-1, ERB-B1 and combinations thereof.

45. The method according to claim 44 wherein the method generates antigen specific cytotoxic lymphocytes to the tumor antigen or antigenic epitopes thereof.

46. A method of making a humanized polynucleotide vector comprising:

operably linking a human derived promoter or mammalian homolog thereof which is functional in a target tissue or target cells to a sequence acceptance site, said site directionally accepts cDNA target products from rtPCR cloning via unique sites within an interrupted palindrome recognition sequence for a restriction endonuclease, said vector lacking nucleic acid sequences encoding vector-derived polypeptides wherein, said vector lacks an antibiotic resistance encoding nucleic acid sequence.

47. The method according to claim 46, wherein the human derived promoter is a RANTES promoter or portion thereof.

48. A isolate antibody comprising an antibody elicited in response to immunization with the polynucleotide vector vaccine according to claim 17-22 or 23, said antibody is specific for the target antigen or antigenic epitope thereof expressed by the mammalian target tissue or mammalian target cell.

50. The sequence acceptance site comprising nucleic acid sequences which accept cDNA target products from rtPCR cloning wherein the sequence acceptance site directionally accepts target sequence specific products from rtPCR cloning via unique sites within an interrupted palindrome recognition sequence for a restriction endonuclease.

51. The sequence acceptance site according to claim 50 wherein the restriction endonuclease is Bgl I.

52. The sequence acceptance site according to claim 50 or 51 wherein

a 5' acceptance site reads on the positive strand as GCCACCATGGCC.

53. The sequence acceptance site according to claim 52 wherein a 3' acceptance site reads on the positive strand as GCCTTAAGGGC.

54. The sequence acceptance site according to claim 50 wherein the site comprises the nucleotide sequence as depicted in Figure 2.

55. A use of a polynucleotide vector vaccine in the manufacture of a medicament for use in a method of stimulating a specific immune response to at least one target antigen or antigenic epitope thereof in a mammal, said method comprising:

administration of an effective amount of a polynucleotide vector vaccine according to claims 17-22 or 23 into the mammal, said amount elicits the specific immune response to the target antigen or epitope thereof.

56. A use according to claim 55, wherein a site of administration is muscle or skin.

57. A use according to claim 55 or 56 further comprising an expression enhancing agent.

58. The use according to claim 57, wherein the expression enhancing agent is a mycotoxic agent.

59. The use according to claim 58, wherein the mycotoxic agent is bupivacaine-HCl or dextrose.

60. The use according to claims 55-58 or 59 wherein the target antigen is a tumor antigen, bacterial antigen, viral antigen or parasitic antigen.

61. The use according to claim 60, wherein the tumor antigen is selected from the group consisting of p53, RB, ras, int-2, Hst, Tre 17, BRCA-1, BRCA-2, MUC-1, HER-2/neu, truncated EGFRvIII, CEA, MyC, Myb, OB-1, OB-2, BCR/ABL, GIP, GSP, RET, ROS, HS, SRC, TRC, WT1, DCC, NF1, FAB, MEN-1, ERB-B1 and combinations thereof.

62. The use according to claim 61, wherein the method generates antigen specific cytotoxic lymphocytes to the tumor antigen or antigenic epitopes thereof.

64. The humanized polynucleotide vector according to claims 1-6 or 8-16, wherein the recognition sequence is recognized by Bgl I restriction endonuclease.

65. The humanized polynucleotide vector according to claim 8, wherein the

nucleic acid sequence which allows for selection is a suppressor tRNA gene, a synthetic SupF complementation tRNA gene, or functional derivatives thereof.

66. The humanized polynucleotide vector according to claim 65, wherein the nucleic acid sequence is selected from the group consisting of SupE, SupP, SupD, SupU, SupF, SupZ, glyT, glyU, SerP, psu^{+}, psu^{+}-C34, psu^{+}AM and psu^{+}OC.

67. A polynucleotide vector according to claims 1-6 or 8-12 wherein a 3' sequence acceptance site reads on the positive strand as GCCTTAAGGGC.

68. The humanized polynucleotide vector according to claims 1-6 or 8-13 wherein the sequence acceptance site comprises the nucleotide sequence as depicted in Figure 2.

69. The method according to any of claims 24 through 27 wherein the method is ex vivo.